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Antileishmanial Activity of an Indole Alkaloid from *Peschiera australis*

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In this study, we show the leishmanicidal effects of a chloroform fraction (CLF) and a purified indole alkaloid obtained from crude stem extract of *Peschiera australis* against *Leishmania amazonensis*, a causative agent of cutaneous leishmaniasis in the New World. In a bioassay-guided chemical fractionation, the leishmanicidal activity in CLF completely and irreversibly inhibited promastigote growth. This fraction was also active against amastigotes in infected murine macrophages. Chemical analysis of CLF identified an iboga-type indole alkaloid coronaridine as one of its major compounds. Coronaridine showed potent antileishmanial activity, inhibiting promastigote and amastigote growth. Promastigotes and amastigotes treated with CLF or coronaridine showed pronounced alterations in their mitochondria as assessed by transmission electron microscopy.

Leishmaniasis is a major health problem that affects approximately 12 million people worldwide, with 2 million new cases diagnosed every year (26). The causative agents of this disease are parasites of the genus *Leishmania*, which infect and replicate in macrophages of the vertebrate host. Leishmaniasis presents a broad clinical spectrum, ranging from asymptomatic and self-healing infections to those causing significant mortality (1). Recently, a dramatic increase in the number of cases of leishmaniasis has been observed in patients with compromised T-cell function, such as those infected with the human immunodeficiency virus (25).

Pentavalent antimonials are still the first choice among drugs used for the treatment of leishmaniasis. In general, these compounds are toxic and expensive, and they require long-term use during treatment. Recently, the emergence of antimony-resistant parasites has been reported (1, 10, 14), which has compelled the search for new antileishmanial agents.

Several new antileishmanial compounds are under development, but a drug with the capacity to completely cure these infections has not been discovered. Although most active drugs against infectious agents are derived from medicinal plants, medicinal scientific evaluation of the medicinal properties of plants remains grossly understudied.

Among all families of the plant kingdom, members of the Apocynaceae family have been used for centuries in folk medicine, and many of their compounds have been isolated and are now in clinical use as separate drugs, such as vinblastine, vincristine, and reserpine (18). Among the members of the Apocynaceae family, the genus *Peschiera* has been used in Brazil, while the synonymous genus *Tabernaemontana* has been used in Central and South America, and the genus *Ervatamia* has been used in Australia and Asia. Ethnobotanical sources mention that the most common medicinal uses of this genus involve its antimicrobial action against infectious diseases such as syphilis, leprosy, and gonorrhea, as well as its antiparasitic action against worms, dysentery, diarrhea, and malaria (22). Anti-inflammatory, antitumor, and analgesic activities have also been reported (19, 20, 22). The effective uses described in folklore are probably based on the presence of indole alkaloids, which are the main secondary metabolites in this genus. One of these alkaloids, olivacine, has shown strong activity against human carcinomas (16) as well as parasites (8, 13, 27). Antileishmanial activity has been reported for bis-indole alkaloids isolated from *Peschiera van heurkii* (17).

The species *Peschiera australis* (Müll. Arg.) Miers, which flourishes in Brazil, Argentina, Uruguay, and Paraguay, has been poorly investigated with regard to its chemical composition and specific pharmacological activities. The study reported here was undertaken to examine the potential antileishmanial activity of *P. australis*. We found that an ethanolic extract from the stems of *P. australis* inhibited the growth of *Leishmania amazonensis* promastigotes in axenic cultures and of amastigotes in infected murine macrophages. A compound purified by a bioassay-guided chemical fractionation of this extract was identified as the indole alkaloid coronaridine, which exhibited potent antileishmanial activity (2).

MATERIALS AND METHODS

Plant extraction and fractionation. Stem material at the secondary stage of growth was collected from two specimens of *P. australis* in the Botanical Gardens of Rio de Janeiro (Rio de Janeiro, Brazil), washed, dried at 45°C for 7 days, and ground in an industrial blender. The crude extract was prepared by soaking the material (350 g) with ethanol (Reagen, Rio de Janeiro, Brazil), in a Soxhlet apparatus at 78°C for 36 h. The ethanolic extract was dried, suspended (80% [wt/vol]) in 5% HCl (Reagen), and partitioned with hexane (3× 250 ml) (Rea-

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gen) and chloroform (3× 250 ml) (Reagen). The fractions were concentrated in a rotating evaporator (Fission, São Paulo, Brazil), suspended in bidistilled water, lyophilized, and stored at −20°C until used. Organic fractions were diluted in dimethyl sulfoxide (DMSO) (Sigma Chemical Co., St. Louis, Mo.) for the biological assays. Promastigote growth and amastigote survival inhibition assays were used to test the crude extract fractions (hexane, chloroform, and aqueous fractions) and coronaridine for antileishmanial activity. Coronaridine was purified as previously described by Rates et al. (19).

Structure elucidation. As the biological activity was present in the chloroform fraction (CLF), its composition was further analyzed by high resolution (HR) gas chromatography-mass spectrometry (GC-MS), using an HP 5890-a spectrometer equipped with flame ionization detection (290°C), column 25 m, 0.25-μm film thickness, and 0.25-mm fused silica capillary column DB-5 (J&W); a flow rate of 1.2 ml of He per mm; a temperature program of 150°C, rate 4°C/min, to 290°C for 5 min and with the injection port in split mode 1:20 at 240°C. The integrator was model HP 3395. GC-MS was performed under the same conditions in an HP 5970 spectrometer with ionization energy of 70 eV and an ion source at 280°C.

Coronaridine was identified as the major constituent in CLF. Its presence was confirmed by coinjection of coronaridine standard with CLF in two distinct phase capillary gas chromatography columns.

Parasite culture. *L. amazonensis* (WHOM/BR/75/Josefa) promastigotes were cultured at 26°C in SIM-F (Schneider insect medium [Sigma] plus 10% fetal calf serum [FCS] [Gibco-BRL, Gaithersburg, Md.] and 15 μg of gentamycin per ml) (Schering-Plough, Rio de Janeiro, Brazil).

Antipromastigote activity. Promastigotes were incubated in SIM-F in the presence of different concentrations of the ethanolic extract, the CLF, or purified coronaridine, which were added only once to the cultures. After 3 days at 26°C, parasite survival was estimated by counting viable or motile forms in a hematocytometer. In all tests, 1% DMSO (a concentration five fold higher than that used to dissolve the higher dose of the compounds) and medium alone were used as controls. To investigate the reversibility of the compounds/effects, promastigotes in SIM-F were treated with CLF at 100 μg/ml for 1 h or 20 μg/ml for 24 h. The parasites were then washed and incubated in fresh medium, and their viability was estimated each 24 h. All cultures were performed in triplicate, and the results were expressed as percent growth in comparison to that of the controls.

Antiamastigote activity. Resident peritoneal cells from normal BALB/c mice were harvested in RPMI 1640 medium (Biochrom KG, Berlin, Germany) plus 15 μg of gentamycin per ml. Cells were plated onto 13-mm^2 coverslips (Thomas Scientific, Swedesboro, N.J.) inside 24-well plates (Nunc, Roskilde, Denmark) and allowed to adhere for 2 h at 37°C in 5% CO_2. Nonadherent cells were removed, and macrophages were incubated overnight in RPMI supplemented with 10% FCS as described above. Adhered macrophages were infected with *L. amazonensis* promastigotes (stationary growth phase) at a parasite/macrophage ratio of 6:1 and incubated at 37°C in 5% CO_2. After 1 h of incubation, free promastigotes were removed by extensive washing with phosphate-buffered saline (PBS) (0.01 M), and the cultures were incubated for 4 days as described above. Treatment of infected macrophages with crude extract, fractions, and purified coronaridine was done by following two different protocols: (i) one treatment 1 h after the infection and (ii) addition of the compounds once a day for 3 days postinfection without replacing the culture medium. Glucantime (Rhodia, Sao Paulo, Brazil) was used as a control for parasite growth inhibition. After 4 days, the monolayers were washed with PBS at 37°C, fixed in methanol, and stained with Giemsa. The number of amastigotes was determined by counting at least 400 macrophages in duplicate cultures, and results were expressed as percentage of survival in comparison to that of the controls. The survival indices were obtained by multiplying the percentage of infected macrophages by the number of amastigotes per infected macrophage. Only experiments with a survival index of ≥220 for the untreated macrophages were considered.

Ultrastructural analysis. *L. amazonensis* promastigotes (10^8) treated with 100 μg of the CLF per ml or with medium alone for 1 h at 26°C were washed in PBS and fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodilate buffer (SCB) at 4°C. The parasites were washed three times with 0.1 M SCB and postfixed with 1% osmium tetroxide in 0.1 M SCB plus 0.8% potassium ferricianide and 5 mM calcium chloride for 60 min at room temperature. After dehydration in acetone, the material was incubated in an acetone-epon mixture (1:1) at room temperature for 24 h and then transferred to pure epon at 60°C for 72 h. *L. amazonensis*-infected resident mouse peritoneal macrophages in 25-cm^2 cell culture flasks (Nunc) were treated with 20 μg of the CLF or coronaridine per ml for 10 h at 37°C in 5% CO_2. CLF and coronaridine effects on noninfected macrophages were also evaluated, and 1% DMSO was used as a control in both situations. The cells were processed for transmission electron microscopy as described above. Sections obtained in a Reichert Ultracut (Leica, Nussloch, Germany) were stained with uranyl acetate and lead citrate and were examined in a Zeiss 900 transmission electron microscope (Carl Zeiss, Oberkochen, Germany).

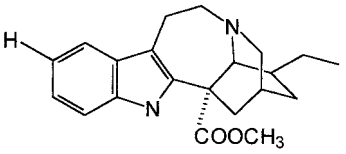

FIG. 1. Coronaridine, one of the indole alkaloids found in the CLF from *P. australis*.

Phagocytosis assay. Resident mouse macrophages were treated with 20 μg of either coronaridine or Glucantime per ml for 24 h. Stationary-phase *L. amazonensis* promastigotes used as corpuscular stimuli were added to monolayers at a parasite/macrophage ratio of 6:1, and the preparations were incubated at 37°C in an atmosphere of 5% CO_2. After 1 h, monolayers were washed, fixed, and Giemsa stained as described above. Examing 400 cells at random in duplicate cultures, we determined the percentage of macrophages with adhered or phagocytosed promastigotes. Results were expressed as percentage phagocytosis in comparison to that of the controls.

Cytotoxicity assays. Resident mouse macrophages adhered to 24-well plates were treated with coronaridine and Glucantime at the indicated concentrations for 24 h at 37°C in 5% CO_2. The macrophages were than washed and incubated with 0.3% trypan blue solution, and the number of viable cells was scored in an inverted microscope. This test was also used to check drug cytotoxicity in monocyte-derived macrophages (MDM) obtained from three healthy donors according to the method of (R. G. Lima, E. M. B. Saraiva, J. Van Weyenbergh, M. Barral-Neto, G. Galvão-Castro, and D. C. Bou-Habib, submitted for publication).

Additionally, drug citotoxicity to human MDM was determined by the 2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5 carboxanilida (XTT, Sigma) method described by Bou-Habib et al. (3). For both tests using human MDM, the cells were treated with a single dose of coronaridine, and their viability was checked after 7 days of incubation at 37°C in 5% CO_2. Results were expressed as percentage of viable cells in relation to viable cells of untreated controls.

Nitric oxide production. Adhered J774.A1 murine macrophage cells (10^6 cells/well in a 24-well plate) were not activated or were activated with 10% γ-interferon (γ-IFN, L1210 cell line, 4-day culture supernatant) and 100 ng of lipopolysaccharide per ml from *Escherichia coli* O111:B4 (Difco Laboratories Inc., Detroit, Mich.). After 24 h at 37°C in 5% CO_2, the monolayers were treated with 10 and 20 μg of CLF or coronaridine per ml. The nitrite concentration in the culture medium was assayed by the Griess reaction (9). Plates were read at 490 nm, and the NO_2^- concentration was determined with reference to a standard curve, using sodium nitrite. The results were expressed as micromolar concentrations of nitrite.

Statistical analysis. Results were statistically analyzed by the Student's *t* test. *P* values of ≤0.05 were considered significant. The 50% inhibitory concentration (IC_{50}) was determined using MATLAB software (Mathworks, Inc., Natick, Mass.) with a specific toolbox for estimating curves.

RESULTS

Partitioning the crude ethanolic stem extract of *P. australis* with organic solvents yielded three fractions: hexane, chloroform, and aqueous fractions. All of these fractions were assayed for antileishmanial activity against promastigote and amastigote forms of the parasite. CLF was the only fraction that showed significant antileishmanial effect on both forms of the parasite. The HRGC-MS analysis revealed that CLF was rich in alkaloids, mainly indole alkaloids, and that coronaridine was one of the major components identified (Fig. 1). This alkaloid was first isolated and characterized by Gorman et al. (7) from *Tabernaemontana coronaria* (syn. *Ervatamia coronaria*). It was classified as an Iboga-type indole alkaloid because it has an isoquinuclidine ring fused to an indole moiety (18).

Antipromastigote activity. Daily treatment with 1 mg of the crude extract per ml inhibited the growth of the *L. amazonensis* promastigotes. An inhibition of 30% in the parasite growth and

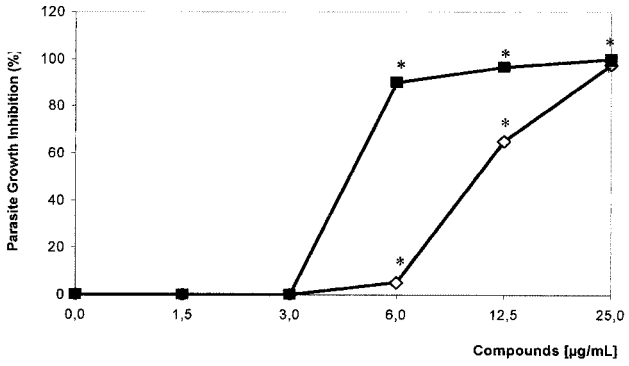

FIG. 2. Inhibition of *L. amazonensis* promastigote growth by *P. australis* CLF (diamonds) and coronaridine (squares). The parasites were treated only once with the different compounds, and their growth was assessed 3 days later. The results are from 1 of 3 experiments done in triplicate, and they are shown as percentage inhibition of parasite growth, measured by counting motile/viable promastigotes. *, $P < 0.05$.

motility was observed after 24 h, reaching 100% inhibition after 6 days of treatment (data not shown). Treatment of promastigotes with the aqueous fraction (100 μg/ml) inhibited the parasite survival by 80% after 3 days.

A dose-dependent antipromastigote effect of CLF and purified coronaridine is shown in Fig. 2. A 97% inhibition of promastigote growth was obtained with 12.5 μg of coronaridine per ml, while CLF at the same concentration inhibited 65% of growth. The reversibility of the effect on promastigotes was tested by incubating parasites with CLF at different times, followed by culture in fresh medium. Treatment with 100 μg of CLF per ml reduced parasite growth by 43% in the first hour, reaching 100% lethality after 24 h. Treatment with 20 μg of CLF per ml reduced parasite growth by 75% after 24 h, reaching 100% after 72 h of incubation in fresh medium (data not shown). This indicated that CLF induced irreversible damage in the promastigotes' ability to replicate in culture medium.

Antiamastigote activity. The leishmanicidal activity of CLF and purified coronaridine was evaluated in *L. amazonensis*-infected macrophage cultures by adding the compounds in the first day of culture or once a day for 3 days. The extract and the compounds were added to the cultures without replacing the medium. The stem crude extract inhibited parasite survival by 70% in both protocols (data not shown). The effect of the drugs on amastigote-infected macrophages treated once for 1 h after infection is shown in Fig. 3. A 40% inhibition of amastigote survival was seen using 1 μg of CLF per ml, and 10 and 20 μg of CLF per ml inhibited parasite survival by 82 and 98%, respectively. When coronaridine was used at 10 and 20 μg/ml, the amastigote survival decreased to 38 and 79%, respectively. IC_{50}s of 2.6 and 12 μg/ml were calculated for CLF and coronaridine, respectively. When the monolayers were treated with 10 or 20 μg of Glucantime per ml, an inhibition of 36 or 70%, respectively, was observed with an IC_{50} of 15 μg/ml.

A stronger inhibitory effect on the amastigote survival was observed when the infected macrophages were treated three times with the drugs (Fig. 4). CLF induced a 49% inhibition at 1 μg/ml, reaching 90 and 99% with 10 and 20 μg/ml, respectively with an IC_{50} of 1.25 μg/ml. The treatment with coronaridine inhibited amastigote survival by 66% at 10 μg/ml and by

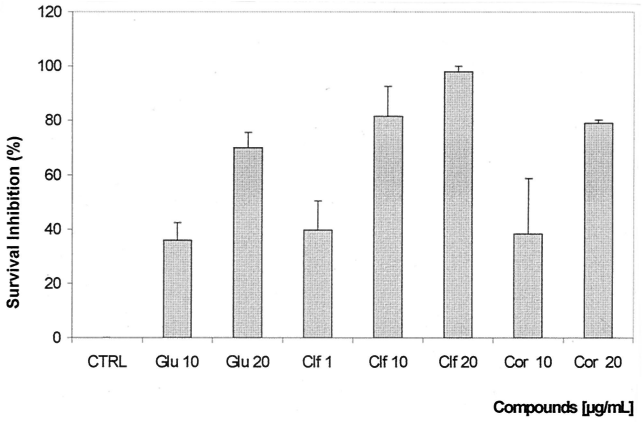

FIG. 3. Effect of *P. australis* CLF (Clf), coronaridine (Cor), and Glucantime (Glu) on amastigote survival. *L. amazonensis*-infected mouse peritoneal macrophages were treated with different concentrations of the drugs 1 h after the infection, and the amastigote survival was assessed 4 days later. Results from four experiments in duplicate are shown as percentage ± standard deviations of survival inhibition in relation to untreated control. All the results were significant ($P < 0.05$).

85% at 20 μg/ml (IC_{50}, 4.7 μg/ml). Glucantime decreased the amastigote survival by 62% at 10 μg/ml and 87% at 20 μg/ml (IC_{50}, 6.6 μg/ml).

Ultrastructural effects on promastigotes, amastigotes, and host cells. Electron microscopy studies were done to determine the ultrastructural changes in the parasites and host cells treated with the drugs. Promastigotes treated with 1% DMSO showed no morphological differences from untreated controls (data not shown). The initial morphological alteration detectable in promastigotes exposed to CLF (100 μg/ml for 1 h) was in the mitochondria, which showed alterations similar to those of the treated amastigotes (Fig. 5B, D, and E).

Uninfected murine peritoneal macrophages treated with 20 μg of CLF or coronaridine per ml for 24 h showed a characteristic ultrastructural pattern not differing from that of untreated controls. These uninfected macrophages presented a well-defined nucleus, endoplasmic reticulum, Golgi complex,

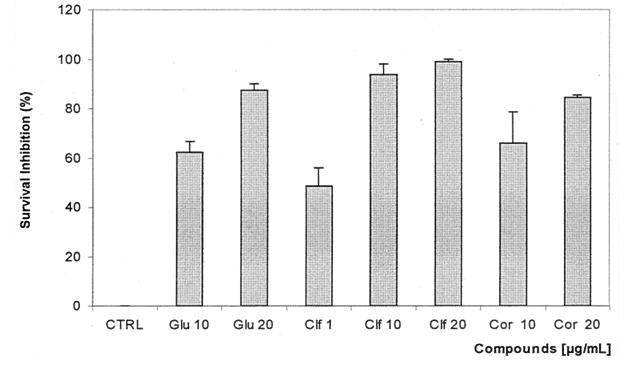

FIG. 4. Effect of *P. australis* CLF (Clf), coronaridine (Cor), and Glucantime (Glu) on amastigote survival. *L. amazonensis*-infected mouse peritoneal macrophages were treated with different concentrations of the drugs 1 h after the infection. Drug treatment was repeated daily during the next 3 days, and amastigote survival was assessed 4 days later. Results are from four experiments in duplicate and are shown as percentages ± standard deviations of survival inhibition in relation to untreated control. All results were significant ($P < 0.05$).

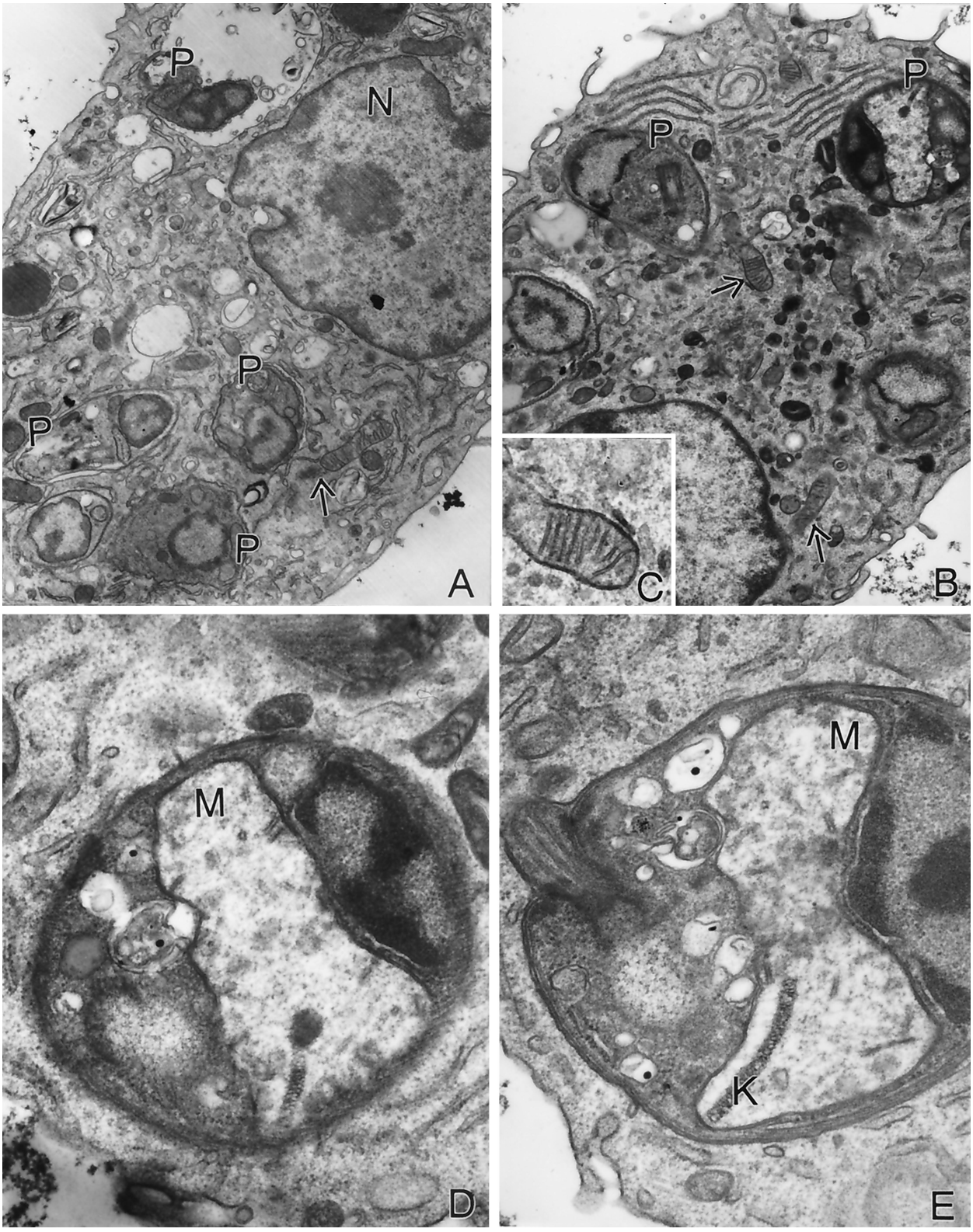

FIG. 5. Ultrastructural effects of coronaridine on intracellular amastigotes. (A) General view of untreated infected macrophages shows a normal aspect. N, nucleus; P, parasites; arrow, mitochondria. Magnification, ×10,000. (B) General view of infected macrophages treated with 20 μg of coronaridine per ml for 10 h, showing parasites in different stages of damage. Note that the macrophage mitochondria (arrows) show a normal aspect better seen in panel C. P, parasite; arrows, macrophage's mitochondria. Magnifications: B, ×8,000; C, ×30,000 (D and E) Amastigote mitochondria showing a swollen organelle containing fragments of membrane and cristae and remains of the mitochondrial matrix. M, amastigote mitochondria; K, kinetoplast. Magnifications, D, ×30,000; E, ×30,000.

and mitochondria with well-distinguished membranes, prominent cristae, and a dense matrix (data not shown).

An untreated *L. amazonensis*-infected macrophage is shown in Fig. 5A, in which the well-preserved ultrastructural morphology of both amastigotes and macrophages could be observed.

Infected macrophages treated with 20 μg of coronaridine per ml for 10 h resulted in amastigotes with swollen mitochondria and disorganized internal-membrane cristae (Fig. 5B, D, and E). The mitochondrion was filled with an amorphous material; however, the kinetoplast DNA appeared normal (Fig. 5D and E). It is important to note that after this treatment macrophages presented well-preserved mitochondria in contrast to the damaged parasite mitochondria (Fig. 5C insert). The same alterations were observed when infected macrophages were treated with 20 μg of the CLF per ml for 10 h. The mitochondria seem to be the first target organelles for both coronaridine and the CLF. Infected macrophages treated with coronaridine or the CLF for 3 days showed many debris-filled vacuoles in which it was impossible to identify intact amastigotes. Macrophages presented normal mitochondria even after these prolonged treatments (data not shown).

Effects of coronaridine on macrophages. In order to test the safety of coronaridine on mammalian cells, murine and human macrophages were treated with the drug, and their viability was checked by trypan blue dye exclusion. When murine macrophages were treated with 20 and 10 μg of coronaridine per ml for 24 h, 83 and 93% of cells, respectively, remained viable. When treated with Glucantime at 20 μg/ml 89% of cells remained viable. Human macrophages treated with 20 μg of coronaridine per ml for 7 days resulted in less than 5% cell death. Further experiments were carried out to determine the potential toxicity of the drug on human macrophages by the XTT method. We observed that 20 and 40 μg of coronaridine per ml resulted in toxicities of 7 and 17%, respectively, to human MDM after 7 days of treatment.

The capacity of the drug to affect macrophage phagocytosis and hence the viability of macrophages was also tested. Murine macrophages treated with 20 μg of coronaridine per ml showed a 15% inhibition of promastigote phagocytosis in relation to that of untreated controls. Glucantime at 20 μg/ml showed a 5% inhibition in the same assay.

Nitric oxide production. To determine whether the inhibition of intracellular parasite growth was due to a general activation of macrophage microbicidal mechanisms, we measured nitric oxide production. Nonactivated J774.A1 macrophages treated for 24 h with 20 μg of CLF or coronaridine per ml were able to produce 24 and 10 μM nitrite, respectively. (Fig. 6). Controls treated with 0.2% DMSO induced 10 μM NO_2^- production. Activation of J774.A1 macrophages with lipopolysaccharide and γ-IFN induced a sixfold increase in their NO_2^- production in comparison with that of nonactivated macrophages. However, addition of 10 μg of coronaridine or CLF per ml to these already activated macrophages did not alter their NO_2^- production (Fig. 6).

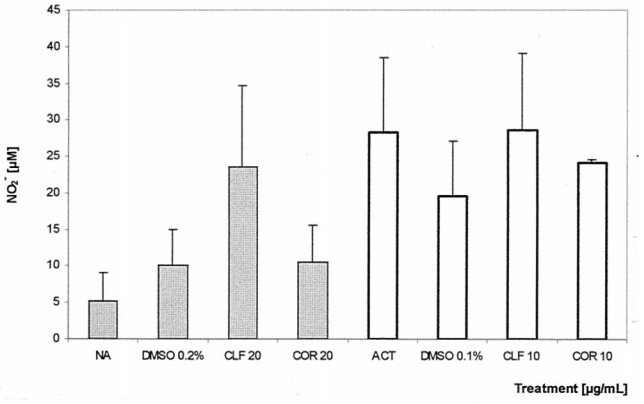

FIG. 6. Effect of CLF and coronaridine on the NO production by nonactivated (gray bars) and γ-IFN-lipopolysaccharide-activated (open bars) J774.A1 macrophages. Supernatants were harvested 24 h after treatment, and NO production was determined by Griess reaction. Data represent the means ± standard deviations of three experiments done in triplicate. NA, nonactivated cells; ACT, activated cells; COR, coronaridine; CLF, chloroform fraction of *P. australis*.

DISCUSSION

Here we describe novel pharmacological activity obtained from extracts of *P. australis* and the pharmacological activity of an indole alkaloid, coronaridine, against promastigote and amastigote forms of the *L. amazonensis* parasite. Our initial observation that the crude extract of this plant inhibited in vitro growth of both developmental forms of *L. amazonensis* (data not shown) prompted us to perform a bioassay-guided fractionation of the antileishmanial activity. This activity was identified in CLF that at 25 μg/ml totally inhibited the promastigote growth (Fig. 2) and at 20 μg/ml inhibited 98% of amastigote survival (Fig. 3). Promastigote damage by CLF was irreversible, suggesting a metabolic injury that could not be reversed by culturing treated promastigotes in fresh medium.

Chemical analysis of CLF identified coronaridine, an ibogatype indole alkaloid, as one of its major constituents. Coronaridine, naturally found in *P. australis* (23), *P. laeta* (28), *P. van heurkii* (17), and *E. coronaria* (11), has also been synthesized and is currently tested as an antiaddictive therapy (6). In our assay conditions, coronaridine (12 μg/ml) showed potent antipromastigote activity (97% killing) and was more active than CLF which at the same concentration killed 65% of the promastigotes (Fig. 2). The contrary was observed with infected macrophages, in which CLF was more active in killing amastigotes than was coronaridine (Fig. 3 and 4). When the IC_{50}s of both compounds were compared, CLF was five- and fourfold more efficient than coronaridine on single- and 3-dose treatments, respectively. Differences could be due to other compounds present in CLF, and the identification and isolation of these compounds are currently being investigated. It is interesting that CLF was six- and fivefold more effective than Glucantime, again based on a comparison of the IC_{50}s of single- and three-dose treatments, respectively.

In order to evaluate the morphological changes induced by CLF and coronaridine, promastigotes and infected macrophages treated with these compounds were analyzed by transmission electron microscopy. The striking ultrastructural change on CLF-exposed promastigotes was the remarkable swelling and disorganization of the mitochondrion. No other changes in parasite organelles were observed in this short treatment. Similar mitochondrial changes were also observed in the intracellular amastigote forms treated with coronaridine (Fig. 5B). It is important that mitochondria, as well as other

organelles of macrophages treated with the same concentration of coronaridine, were not affected, which could suggest the specificity of coronaridine for parasite mitochondria (Fig. 5C, D, and E).

The evidence for selectivity of coronaridine against parasites was reinforced by the preserved mitochondrial activity in drug-treated human macrophages measured by the XTT method. In this assay, mitochondrial dehydrogenases metabolized the XTT reagent to a water-soluble formazan dye (3). Cells with damaged mitochondria were unable to metabolize XTT. Moreover, no other significant damage was observed on coronaridine-treated murine or human macrophages as measured by the trypan blue exclusion test. The phagocytic activity of macrophages was also preserved at coronaridine concentrations toxic to the parasites. In general, a drug may act directly against the parasite or indirectly by activating macrophage mitochondrial mechanisms such as NO production, which has been shown to be the most effective antileishmanial mechanism. Purified coronaridine was unable to up-regulate NO production in either activated or nonactivated macrophages (Fig. 6). The slight stimulation of NO production by CLF could be due to different alkaloids present in this fraction. The pronounced changes in the mitochondria of both forms of the parasite suggest that their energy metabolism was affected. Similar mitochondrial lesions have been shown in *Leishmania major* promastigotes treated with chalcones that inhibited the respiration and mitochondrial dehydrogenases (29). Interestingly, mitochondria are the target organelle in different species of *Leishmania* treated with pentamidine (5, 12), paramomycin (15), licochalcone A (4) and dihydroxy-methoxychalcone (21). It is interesting that ketoconazol, which is an inhibitor of ergosterol synthesis, also affects mitochondrion morphology (24). Ergosterol synthesis is an important chemotherapeutic target, as this lipid is the main component of *Leishmania* membranes. Although we still don't know the mechanism responsible for parasite killing, elucidation of this phenomenon is currently under investigation in our laboratory.

Our results reveal a novel pharmacological activity of coronaridine, besides its antiaddictive property (7). Laboratory synthesis and the possibility to modify coronaridine chemical structure constitute important advantages for development of new antileishmanial therapy.

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